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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/583,277	06/16/2006	Yoshiko Minakuchi	0020-5493PUS1	2615

2292 7590 09/16/2010

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EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
09/16/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/583,277	MINAKUCHI ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 September 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3,5,8-11 and 22-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3, 5, 8-11 and 22-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/7/2010 has been entered.

Claims 1-3, 5, 8-11 and new claims 22-24 are pending in the present application, and they are examined on the merits herein with the previously elected **siRNA as a species of a nucleic acid**.

Response to Amendment

The rejection of claim 7 under 35 U.S.C. 103(a) as being unpatentable over over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), Lam et al (Biochim. Biophys. Acta 1463:279-290, 2000) and Kubota et al. (US 2004/0052840; IDS) was withdrawn because claim 7 was cancelled.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

New claims 22-23 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. ***This is a new ground of rejection.***

Claim 22 recites the limitation "the surface of a cell culture vessel" in line 2 of the claim. There is insufficient antecedent basis for this limitation in the claim. This is because prior to this limitation, there is no recitation of any surface of a cell culture vessel. Therefore, which particular “the surface” do Applicants refer to? For the purpose of a compact prosecution, the examiner simply interprets the above limitation simply to be a surface of a cell culture vessel.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-3, 5 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Graham et al (Virology 54:536-539, 1973; IDS). ***This is a new ground of rejection.***

Graham et al disclose a method of transforming rat embryo and rat kidney cells with human adenovirus type 5 (Ad 5) DNA, said method comprises at least the following steps: (a) diluting the viral DNA in isotonic saline and mixed with 2.5M $CaCl_2$ to a final Ca2+ concentration of 125 mM; (b) incubating the mixture at room temperature for 15-20 minutes prior inoculating the mixture onto cell monolayers, usually in aliquots of 0.5

mL, for 20 minutes at room temperature; (c) the cell culture dish of step (b) received 5 mL of Eagle's basal medium supplemented with 2% heated horse serum (BME + 2% HS); (d) **the BME was supplemented with additional $CaCl_2$ to bring the final Ca2+ concentration (comprising Ca2+ from the inoculum plus that from the medium) to approximately to 15 mM**, if the initial DNA inoculum was less than 0.5 mL (see at least page 536, particularly col. 2).

Since the instant claims encompass the use of **a nucleic acid in any form** (e.g., ss-DNA, ds-DNA, ss-RNA, ds-RNA, peptide-nucleic acid, in a complex or in an inclusion body; see at least Disclosure of the Invention and original claims 2-7), and due to the open language of the term "comprising", the teachings of Graham et al meet all the limitation of the instant claims as broadly written. Please note that Graham et al succeeded to transfer human Ad5 DNA into the tested cells as evidenced by the attainment of foci of transformed cells (Figures 1-2 and Table 1); and the 2.5M $CaCl_2$ solution is a stock solution that was used to attain the desired final Ca2+ concentrations of 125 mM and 15 mM in steps (a) and (d), respectively. Moreover, Ad 5 DNA is a double-stranded linear DNA.

Accordingly, the reference anticipates the instant claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and

> the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5 and 8-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al (Biochim. Biophys. Acta 1463:279-290, 2000) for the same reasons already set forth in the Office action dated 3/4/2010 (pages 3-7). ***The same rejection is restated below.***

With respect to the elected species, Li et al disclose a method for introducing dsRNAs or siRNAs into cells, cell culture, organs and tissues, and whole organisms to attenuate gene expression (see at least the abstract; Summary of the invention, particularly paragraphs 35-54 and claims). Li et al teach specifically that the dsRNA nucleotide sequence is preferably at least about 25 bases and that it can be introduced into a cell in various ways, including liposome-mediated delivery, viral infection, transformation, transfection mediated by calcium phosphate, electroporation among others (paragraphs 39 and 44). In an exemplification Li et al disclose that rat cells were

transfected by **overlaying onto the cells with lipid-DNA complexes containing dsGFP RNA in serum-free DMEM and incubated for 5 hours at 37 ^{0}C, following by the addition of DMEM (1 mL) with 20% FBS without removing the transfection mixture** (see example III on page 12).

Li et al do not teach a cell culture method in which following the step of contacting a nucleic acid with a cell in a medium, further adding into the medium with a volume of a high-concentration solution of a calcium chloride to bring the final concentration of calcium chloride within the range of 7.1 mM-30.1 mM.

However, at the effective filing date of the present application, Haberland et al (BBA 1445:21-30, 1999) already disclosed at least that **the addition of soluble calcium ions (2 mM) or calcium phosphate precipitates to cells after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems, and that calcium ion is not needed for non-viral DNA complex uptake** (see at least the abstract; and particularly Figure 3). They specifically stated "Fig. 3 shows the transgene expression following the different procedures of ECV 304 cells. As a control, the cells were transfected as usual with Ca^{2+} present during transfection for 4 h. The transfection medium was then removed and culture medium without Ca^{2+} added for further 24 h post incubation (a). **Transfection of the cells in the absence of Ca2+** followed by thoroughly washing the cells after the 4 h transfection period to remove the H1-DNA complexes and addition of new culture medium containing 2 mM Ca^{2+} and incubation overnight (c) resulted in transgene expression

similar to that observed in (a). At this Ca^{2+} concentration and incubation overnight the cells remained morphologically healthy" (col. 2 at page 25; and particularly Fig. 3).

Moreover, Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) also demonstrated **the importance of the presence of calcium ions in the post-incubation medium after transfection for overcoming serum inhibition in a polycationic or cationic liposomal gene transfer system** (see at least the abstract and Figures 3-4 and 7). They also stated "Fig. 4 shows the transfection efficiencies of neutral H1-DNA complexes **without Ca^{2+} in the transfection medium** at varying serum concentration under different conditions. When increased serum concentrations were present in the transfection medium and 2 mM Ca^{2+}/0.1 mM chloroquine with 10% serum in the postincubation medium, high transfection efficiencies in the total range of serum concentrations were observed" (col. 2 at page 231; see section entitled " Ca^{2+} is not needed for the complex uptake" and Figure 4).

Furthermore, Lam et al also demonstrated that calcium increases the in vitro transfection potency of plasmid DNA-cationic liposome complexes from 3- to 20-fold in a number of different cell lines; and tested the effect with increasing concentration of Ca^{2+} **(0-100 mM)** using an appropriate volume of the 1 M $CaCl_2$ stock solution (see at least the abstract; sections entitled "In vitro transfection in the presence of Ca^{2+}" and "The transfection potency of complexes is increased in the presence of Ca^{2+}"). Lam et al further stated "**[u]p to 20-fold increases in transgene expression were detected at Ca^{2+} concentrations between 5 and 25 mM. Transfection potencies decreased for Ca^{2+} concentrations at 50 mM or greater, where more than 20% reductions in total**

cellular protein levels were observed, indicating toxicity (data not shown)" (col. 1 at page 282, last paragraph).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to modify the teachings of Li et al at least with respect to a method for introducing dsRNAs or siRNAs into cells in a cell culture to attenuate gene expression by adding an appropriate volume of a high-concentration calcium chloride solution (concentration of 0.1M or greater; see definition of the term "high-concentration" on page 13, lines 16-20 of the instant specification) to the medium to attain at least a final concentration of calcium chloride in the range of 2 mM - 25 mM following the step of contacting a nucleic acid with a cell, in light of the teachings of Haberland et al (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al as presented above. With respect to the limitation of dependent claims 10-11, it would also have been obvious for an ordinary skilled artisan to determine and use an aliquot within the range of 1 uL-20 uL of a high-concentration calcium chloride solution (e.g., 1 M $CaCl_2$ stock solution) per 500 uL of the medium to attain the desired final calcium chloride concentration.

An ordinary skilled artisan would have been motivated to carry out the above modifications because both Haberland et al references demonstrated that at least the addition of soluble calcium ions (2 mM) to cells **after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems; calcium ion is not needed for non-viral DNA complex uptake; and the presence of calcium ions in the post-incubation medium after transfection can overcome serum**

inhibition. Furthermore, Ca^{2+} concentrations between 5 and 25 mM have been used successfully to enhance in vitro transfection potency of plasmid DNA- cationic liposome complexes without toxicity to cells as taught by Lam et al.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Lam et al; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 9/7/2010 (pages 5-6) have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

Applicants argue basically that in all of the cited references the nucleic acid being introduced into the cells is complexed with a further substance, and none of the references teaches or suggests that the nucleic acid should be added to cells without complexation with another substance. Applicants refer the examiner to example 4 and results shown in Figure 4 that show the delivery of a siRNA to cells is enhanced by application of a high concentration solution of calcium chloride without use of any liposome or other complexation of the siRNA.

Please note that the instant claims encompass the use of **a nucleic acid in any form** (e.g., ss-DNA, ds-DNA, ss-RNA, ds-RNA, peptide-nucleic acid, in a complex or in an inclusion body; see at least Disclosure of the Invention and original claims 2-7, **particularly the dependency of original claims 6-7**), not necessarily limited only in the form of the siRNA designated as hEx3.-1 in example 4 as argued by Applicants. The instant specification states explicitly that "The nucleic acid used herein may be in the form of a complex or an inclusion body with a biodegradable substance or a living body-derived substance, as mentioned above. Particularly, such a complex or an inclusion body can be conveniently used in the method wherein an aqueous nucleic acid solution is added to a cell culture vessel and made dry or absorb to the vessel" (page 14, lines 13-18). Additionally, it is noted that the primary Li et al reference states specifically "**In the case of a cell culture or tissue explant, the cells are conveniently incubated in a solution containing the dsRNA or lipid-mediated transfection**" (paragraph 44).

Accordingly, claims 1-3, 5 and 8-11 are still rejected under 35 U.S.C. 103(a) as being unpatentable over the cited prior art references for the reasons set forth above.

New claims 22-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Lam et al (Biochim. Biophys. Acta 1463:279-290, 2000) as applied to claims 1-3, 5 and 8-11

above, and further in view of Sabatini, D (US 2003/0228601). ***This is a new ground of rejection.***

The combined teachings of Li et al, of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al. were presented above. However, none of the cited references teaches specifically a method of nucleic acid transfer comprising the step of absorbing a nucleic acid onto the surface of a cell culture vessel.

At the effective filing date of the present application, Sabatini already taught a reverse transfection method comprising the steps of: (a) depositing a mixture comprising a nucleic acid of interest (e.g., oligonucleotide, DNA, RNA) and a carrier protein (e.g., gelatin or other carrier macromolecule) onto a surface (e.g., bottom of wells of a multi-well plate, slide) in defined, discrete locations and allowed to dry, with the result that the nucleic acid-containing mixture is affixed to the surface in defined discrete locations; (b) eukaryotic cells (e.g., mammalian cells, bacterial, insect and plant cells) are plated onto the surface bearing the nucleic acid-containing mixture in sufficient density and under appropriate conditions (e.g., in DMEM containing 10% heat-inactivated fetal serum with pen/strep) for introduction/entry of the nucleic acid into the eukaryotic cells and expression of the nucleic acid or its interaction with cellular components (see at least paragraphs 4-19). Sabatini also disclosed that the nucleic acid-containing mixture could also comprise an appropriate lipid-based transfection reagent (e.g., EFFECTEE ^{TM}, Qiagen); and that the reverse transfection method could be used in identifying dsRNA constructs which produce a particular phenotype by RNA

interference (paragraph 94). Furthermore, Sabatini taught that the reverse transfection method provided a strategy for high throughput analysis of gene function in cells (paragraph 4).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to further modify the combined teachings of Li et al, of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al. set forth above by also utilizing the reverse transfection approach that is taught by Sabatini for identifying potential dsRNAs or siRNAs constructs which produce a particular phenotype by RNA interference in cells in a high throughput fashion.

An ordinary skilled artisan would have been further motivated to carry out the above modification because the reverse transfection method of Sabatini which involves the step of absorbing a nucleic acid onto the surface of a cell culture vessel provides a strategy for high throughput analysis of gene function in cells, including identifying potential dsRNAs or siRNAs constructs which produce a particular phenotype by RNA interference.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), Lam et al. and Sabatini; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/QUANG NGUYEN/

Primary Examiner, Art Unit 1633